```
   1 GCGGCCGCGT CGACGTCTTT GCTGCCGCAC AGGGAGCAGC AGCAGCCGCC GACCCGATCC
  61 CTTGGGAGCC CACCAAGTGC TGCGCTTGCT TAGCAGCTAC AGGAGCTGCC GCGGGGTTGC
 121 TCCCTGAGGC AGCGTGCATG TATGGTCCGG CAGCCAGCTT GGTGTCGCAG CCGTACTTCT
 181 TGGAAGCGAG AGAGACTGTG GGAGAGCGCA AATCACTCCA GCCGCTTCCA GGGGAGTCTG
 241 GGGACCGCAG GAGCGTTGGA GGCTGCCTGC CGGCATAAAC AGGAACAAGC GCATTCTTAT
 301 TCTTCTGTGG TTGCTGAGTT CTGGCTGCGT TCAAGGGGGT TCACCTCTTC CCCTTCTGGC
 361 GAGTTTTTGC TGCGTCTTTC CCTAAGAAGC AGCGCCACGT GCGTGGCGTG CCTCAGCCTG
 421 ACGCGGTGCA CCTTTTACGT AAGAGCGTCG ATAGCATCGG TCATCTACAG CAGCGTGCTG
 481 CTGCTTCCGT GACCTTTACA CTGCTTGTGG CGGGCCGTCT TGTAGAGGGG CCATCTGCTT
 541 GTTCGCTGCT GGACGCAGAC CCGGCGCCCG ACATTTCCGG CAGCCGGGCA GTTGAGATAA
 601 ACCGGCTGCC CGGTGGCCGT CGAAATTGAA GCAGGATCTC TACAGTAAGG AACAAATCGC
 661 GCTATTTTTA AGGAGTGTGT ATACTTGGGG CGTTACTCGT GAGTATTGCT GATGATGGAC
 721 GTCCGTGTGG GGGGTAAGTA TCGTTTGGGG AGGAAGATTG GGAGCGGATC CTTCGGCGAC
 781 ATCTACCTTG GTACGAACAT CTCAACAGGA GATGAAGTCG CTATCAAATT GGAAAGCGTG
 841 CGGTCTAGGC ATCCACAACT AATCTATGAA AGCAAGCTGT ACAAAATCCT AACGGGTGGA
 901 ATCGGAATCC CGACTCTTTA CTGGTATGGG ATCGAGGGGG ATTACAACGT TATGATTATT
 961 GAGCTTTTGG GCCCGTCTCT TGAGGACCTC TTCAGCATTT GCAACAGAAA GCTTTCTTTG
1021 AAGACTGTTC TGATGCTCGC CGACCAAATG CTAAATCGTA TTGAGTTCGT CCACAGCAGA
1081 CATTTCATCC ATCGAGACAT CAAGCCTGAC AATTTTTTGA TCGGTAGGGG CAAAAAGATG
1141 TCCATTGTTT TTGCTATCGA CTTTGGCCTC GCAAAGAAGT ACAGAGATCC CAGAACACAG
1201 TCCCATATTC CTTATCGAGA AGGGAAGAAC CTGACAGGTA CCGCGAGGTA CGCCTCTGTG
1261 AACACCCACT TGGGAATAGA ACAGAGCAGG CGCGATGATC TGGAAGCGCT CGGCTACGTC
1321 TTAATGTACT TCAACAGAGG TTCCTTACCC TGGCAAGGAT TAAAGGCCAC TACGAAGAAA
1381 GATAAATATG ACAAGATTAT GGAGAAGAAG ATGTCCACCC CTATTGAAGT CCTTTGCAAA
1441 CAATTTCCAT TTGAGTTTAT CACATATCTG AACTATTGCC GGTCTCTGCG ATTCGAAGAT
1501 CGCCCGGACT ATTCCTATTT GAGACGGTTG TTCAAGGATC TTTTCTTCCG TGAGGGATAC
1561 CAGTATGACT TTATATTCGA TTGGACATTT CTGCATGCTG AGAGAGAGCG CGAGCGTCAA
1621 AGACGATCGA TGGTCAACCA AGGCGCAGAA TCAGGGAACC AGTGGAGACG AGACGCGTCG
1681 GGCAGAGATC CACTTGGACG GTTGCCTCAG TTAGAACCGT AATCTCTTTA CGGGCAGATT
1741 GCCGTACGGG TCTTCTGCTC ATTCAGTGGC AGTGCCACCG CAGTGCTATC TGAGGCTGTG
1801 GCTTCAGGAT GTGGTAGCCA GTTACCATGG TCACTTGCCC TCGCTAGGAC AGCCTTCGCA
1861 GGGAAATGTC ACAGTAGCCT GCATTATGTG GTGTGAGAAC TGCTAGCGCA TTCCTGTAGT
1921 TGCTTTTACG AAGCAGGATA CGCAGCGTGC ATCACGCGGT GGTTCGAGCG CTCGCTACGC
1981 ATCACAGGGC TGTGAGGCAA GTTAGTATCT TTGGGGGACG AGTTGAGAGT GTCAGAATCG
2041 ATAGTCTCAG GGCATGCAGG CGAAATGGAG GCTGCGCCAG TAGTGCCAGC CGGTGGCGAA
2101 GGCGTCAAAT TTACTTTTTT TGTTGCTGGG GATATTGTTA GAGCAACAAC TTGGGTCTAG
2161 ATGCTACTGA TAAAAAAAAA AA (SEQ ID NO:1).
```

FIG. 1

```
   1 CCTCGTTTTG CTTCATTCCC CGCCTTTTCT CTGTAGCTAA CCAAAGGAAC AAAGTCAGCG
  61 GTAGAAGCCG TTTCTTCTGT CCGCTTCCCA CTCTTCCCGT TCGGCTGCCC CTGCAGAGCG
 121 CCCTTTCTAT GCGTTGCCAC CCGTCTGCAA GTATCGCGTC TTTCGTCTCA TCAGTGATTT
 181 TCTTTGCGTG TCGCGTTCGG GACGCCCTTT TCTCTCCTCA ACTAACTAGC AGACGTTTCT
 241 TCCGTCCCGC ATGCGACAGC GAAGGGCACG TCCCCCCAGT TCTTCATCGC CCACCTGTTG
 301 TGCAACTTGT CGCCCGTCGT TCTTCACTTC TTCTCTCCCA TCCTCTCGTG ACTCTTCCTC
 361 TCGAGAACTC TTTCTGTCGA ACTCTCAACC CCCACGACTG CTGGTTTCGT GGCCGTCCCG
 421 CATGCACCTT GTGTCCCGCC GCCTTGGCGC AAACACCCGC TTTCTCTGCT GTCCGCCTCC
 481 CGGTGGACTT CTCTCCGTGT TTTTTCGTGT TGCCAAAAGT TTGTCTGCTT TGACGTTTCT
 541 CTGCTCACCC ATTGCCCGCT CTTGATGAGG AACGCTCCAC ATTGACAGCG AACTCACAGC
 601 ACGCACCCTC CGCGAGCGGA CTTTCACGAG CGAGGCAAGA ATCCATCGTC ACCCCGCCTA
 661 CACGTACACT ACTCCACTTG GGTGCCCACG CGCGGCTTCT GGGAGACAGA GACGGTCCTC
 721 GTTTTCCGTG TCAGAACTTT GTCGAGGAAA CGCTGCTGCT GGCAGCGGGG ATTGTGACCC
 781 CCCTCGGCGA ACGGGCGAAG CCGCCCTGTC GCGCGTCGCG ACTCAGCTGA GGCGACAGGC
 841 GGTCGGCGGC GTGACCTCTC TTTCTTTTTG CATTCGGCCC TGATTGCAGC ACGAAGGATG
 901 GAGGTCAGGG TCGGAGGCAA GTACCGACTT GGTCGGAAGA TCGGCAGCGG GTCATTCGGT
 961 GATATTTATA TCGGTGCAAA CATTTTGACG GGGGATGAGG TGGCGATCAA GTTGGAGTCT
1021 ATCAAGTCGA AGCACCCGCA GCTGCTCTAT GAGTCGAAGC TGTACAAACT GCTGGCTGGC
1081 GGCATTGGGA TTCCCATGGT CCACTGGTAC GGCATCGAAG GAGACTACAA TGTTATGGTT
1141 ATCGACCTTC TCGGCCCTTC TCTGGAGGAC CTTTTCAGTA TCTGCAATCG CAAACTCTCT
1201 CTCAAGACGG TGTTGATGCT CGCAGACCAG ATGCTCAACC GCATCGAGTT TGTCCATAGC
1261 AAGAACTTCA TCCATCGCGA TATCAAACCC GACAACTTCC TCATTGGCCG TGGAAAGAAG
1321 ATGTCCGTCG TCTACATCAT CGATTTCGGT TTGGCAAAGA AATATCGAGA CCCAAAGACT
1381 CAGCAACATA TCCCATACAG GGAAGGCAAG AACCTAACAG GCACAGCGCG TTACGCTTCC
1441 ATCAACACCC ACCTGGGGAT CGAGCAGAGT CGGCGAGACG ACCTAGAGGC GCTCGGTTAC
1501 GTTCTCATGT ACTTCAATAG AGGTTCTCTT CCGTKGCAGG GTCTGAAGGC GACGACGAAG
1561 AAGGACAAAT ACGACAAGAT TATGGAGAAG AAAATGTCTA CTCCCATCGA AATTTTGTGC
1621 AAGCATTTCC CATTCGAGTT CATCACCTAC TTGAATTACT GCCGGTCCCT GCGCTTCGAG
1681 GATCGTCCTG ACTACGCATA CTTGCGACGC CTGTTCAAAG ACTTGTTTTT TAGAGAGGGA
1741 TATCAGTACG ACTTCATCTT CGACTGGACT TTCATCAACA CGGAGAAGGA TCGCGCGAGT
1801 CGAAGAAGCC AGCAAGTTTA TGTGGAAGAC AACCGGCAAG TTGAGGAGAA TCAGAACGAG
1861 TTGCCGATGT AGGGTGGTCG GTGTGCGGAG GCCGGCGGGG AGCGTGGAGT CCGCTGAGTC
1921 TGGAAGTCTG CAGACTGTGC TCTGGCACTC GACCCACTTG TTTGTGTTTC CCTCGACTCG
1981 CGCAGGTCGA GGAAAACAGA GACGAACAGG TTACCCAGGA GTGTTTTTGG TCAGGACGCG
2041 CGTCTCCCTC TGAGTTTCGC AAAGTTGCCC CTGGAA (SEQ ID NO:3).
```

FIG. 2

```
   1 TTAACCCTCA CTAAAGGGAA CAAAAGCTGG AGCTCCACCG CGGTGGCGGC GCACCGAGGA
  61 AAACGCAGCT CGTAAGAGAC AGTTCTCTCG GTGAGAAGAG CTATCCGAGA AGGACACCAT
 121 GGCGCACCAT CAAGACACCC GCAACCACAC GGGGGTCGGA CCCTCTTCGT CTATCCCTCT
 181 GAAAGATTTG AAGATCGCCG GCGTCTGGAA AATCGGCAGA AAAATCGGAT CCGGTTCCTT
 241 CGGCGACATA TACAAAGGCC TGAATTCTCA GACCGGTCAG GAGGTGGCGC TGAAGGTCGA
 301 AAGCACCAAG GCGAAGCATC CGCAGTTGCT GTACGAATAC AAACTTTTGA AGCATTTGCA
 361 GGGAGGAACG GGCATTGCTC AAGTGTTCTG TTGCGAGACT GCGGGCGACC ATAACATCAT
 421 GGCCATGGAG TTGCTCGGAC CTTCTTTAGA GGACGTCTTC AACTTGTGCA ATCGCACCTT
 481 CTCTCTCAAA ACCATTCTTC TTCTCGCCGA CCAGTTTCTG CAACGCGTCG AGTACATCCA
 541 CTCCAAGAAT TTCATTCACA GAGATATCAA ACCAGATAAC TTTCTTCTCG GCGGTGCCGG
 601 CAATCAAAAC ACGATCTACG TGATCGACTT CGGCCTGGCG AAGAAGTTTC GCGATCCGAA
 661 AACGCACCAA CATATTCCGT ACAGAGAAAA CAAGAATCTC ACGGGAACGG CGCGCTACGC
 721 GTCCATCAGT GCGCATCTGG GTTCCGAGCA GAGTCGCCGA GATGACCTCG AAGCAGTCGG
 781 CTACGTTCTC ATGTACTTCT GTCGAGGAGG CACGCTGCCT TGGCAGGGCA TCAAAGCGAA
 841 TACCAAACAG GAGAAGTACC ACAAGATCAT GGAGAAGAAG ATGTCGACGC CCGTCGAGGT
 901 GCTATGCAAG GGATATCCAA GCGAATTTGC CACATACTTG CACTACTGCC GCTCCTTGCG
 961 ATTCGAGGAC CGACCGGACT ACGCCTACCT CAAGCGACTC TTTCGAGATC TCTACATCAA
1021 AGAGGGCTAC GATGACAGTG ACCGCGAATT CGACTGGACA GTGAAACTTT CGTCGCGCAG
1081 TCTCGGACCG CCAAGCAGTC GAGCGCAACA TGTTTTACTG AGTCAAGACA CCCGAACGCG
1141 AGGGAAGCGG GAGACAGATC GACCTGTCGC TGCGCGGAGT GGCGACCGCG AACGAGGAAT
1201 CCATTTCAGC AACGGGAACG TGGGCAATCC TTCGATGGCA ACGAACCCCG GCGGCCTGTC
1261 AGTCATGGTG CATGAACGCA CGAGTCTGGT GGATCAGGGA GACCGTGGGT CGCGCGAAAC
1321 TTCTACGCGG AAAGAAGACG CGAAGGACGG CAGATGGCCA GGAGGCAGAT TTTCTTGTCT
1381 TCCACTGTTA TGTCGGCGCT CTCCGACGAA GGCCTAGATG AACTGCGGAG GCGCTCCTGT
1441 CCCCGCAGTT GGCATCTCTC TCCTTCATTG TCGTTGTTCC CCTGCAACTC GAGTCCACCC
1501 TTGACATCCT CGTCTCTCTC TTCCTGTCGG TTTCCTCTTT CTCGTCCTCT CCCCCCTAGC
1561 TTCGTTCTCT CCTTTCTATC CTGCTTCGGC GTCGCCTCAC TTCTCTCCTC ACTTCTCTCC
1621 CTTTTGTTTT TCTTCGCGGC GTCTCTCCTT CACTCTGTCT CCGCCTCTGA CGCCGCGCGG
1681 GAGCCGTTTC CTGCAGGCAG CTCAGGCAAT ACCTGCCTGC AGGTGCCTCT CCTTTTTGAG
1741 CGTCTCTCTT TCCTCGTCGA AACGGTCCTC ACAGCTTCCT CTCCCTGGGG ACGCCGTGGG
1801 CGTAAGTTCT TTTTTTGACG GTCCCGGTGG GCTGGCGTTG TTCGCCTGCC TTCCGCGCAT
1861 GCACTCCGAG CATTTTTGCC TGGCCTGGAC TTCTCCGAGC GAGAGTTGCG GTTTGGCTTC
1921 TGCATCGTCT CCTGCGCTGC TTTCATTTCT CTAGGTTTCT GCTTGCGGCC TCCGTGTACA
1981 GAAATCGGAA GGTGAAGGCG TAGTGGCCAG AGAACGAAGC AAACGAGAGA ACCACGTTCC
2041 ACCTTGTGCG CACGCATGCA TCTACGCATG CACGGTATTT AAGCCGATTT TTTGTGTATG
2101 TATATAGATG TATATATATA TGTATCTACA TGTATCTACC TATATATATG TGTGTGTGTA
2161 AGTGGAAGTG TATTTTTGCA TGTGCAGAAA GCTTTCTTTT CCGCTGGCAT GCTGGAAGAA
2221 GGGCAGGAGG CGACGATCCT GCGAGTCAGG GCGTTCCCTT GTTTCCAGTG AGTTAACCGA
2281 ATTGTTTATT GATATGCGTT TGCATGCATC GACAATGGAT CCTAGACACG CCCGTTTAAA
2341 ATCAGAGGTA TTCCTAAAAA AAAAAAAAAA AAA (SEQ ID NO:5).
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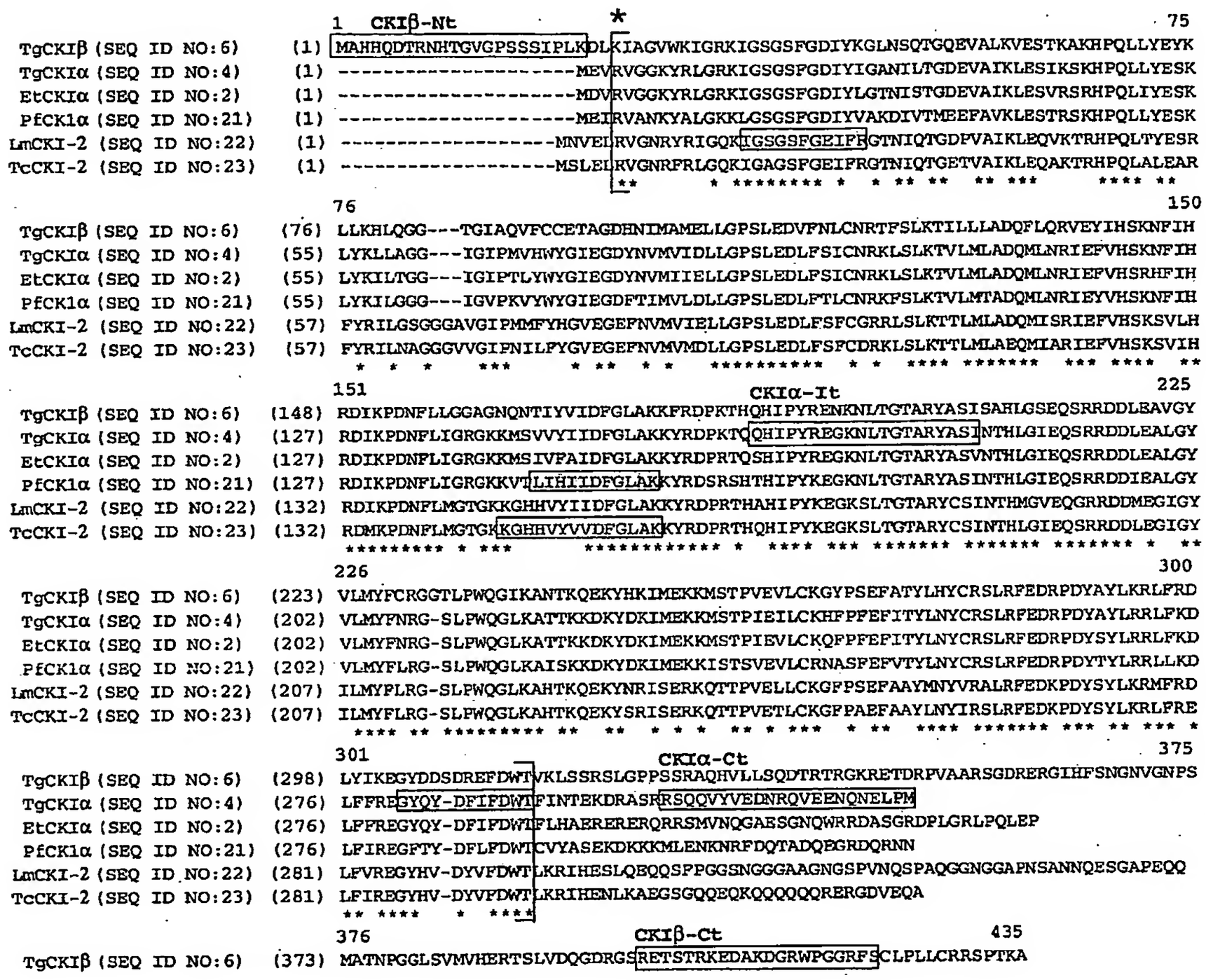
FIG. 3



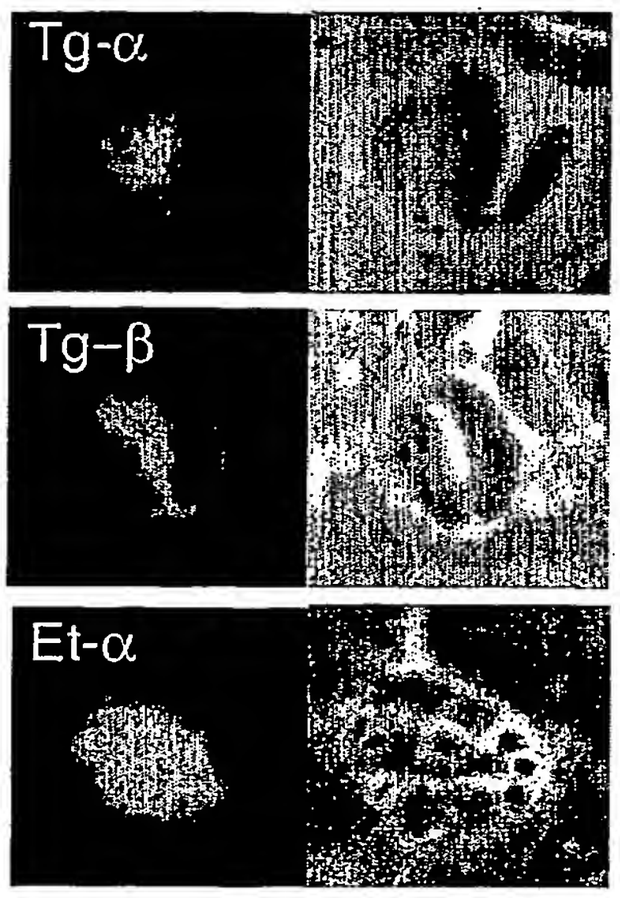
*Brackets mark the catalytic core region

FIG. 4

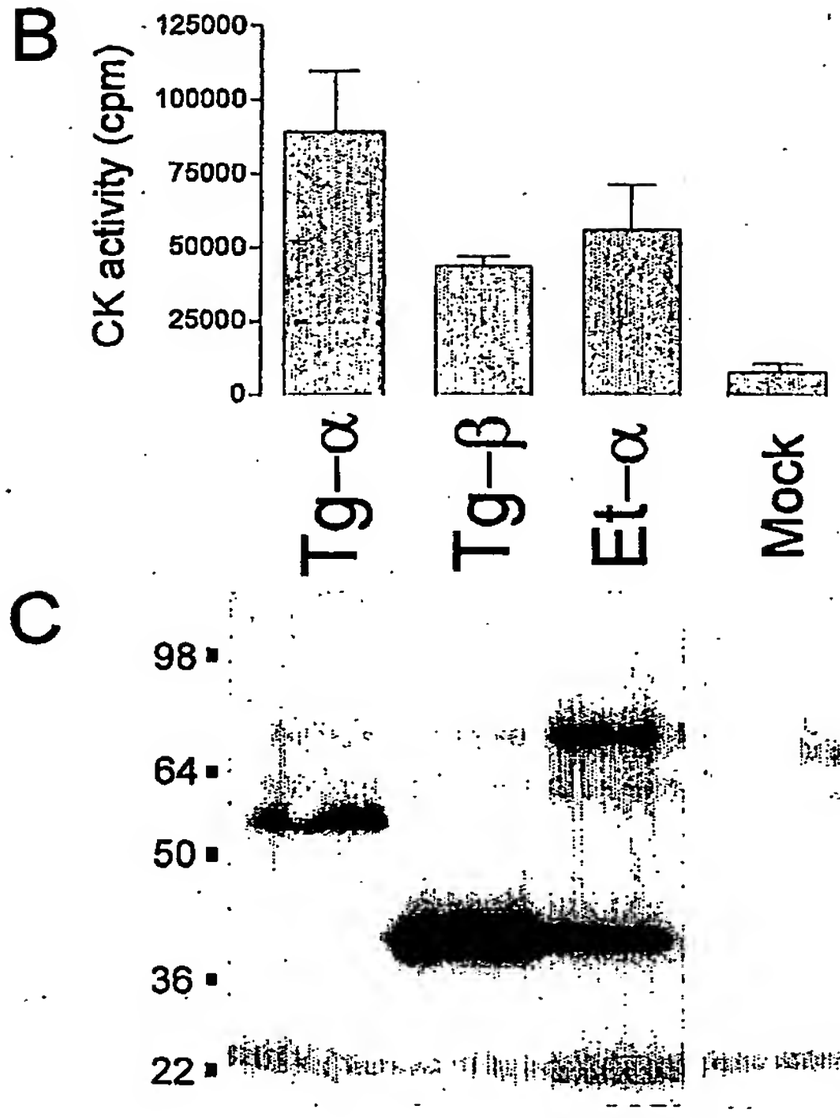
	TgCKIβ	TgCKIα	EtCKIα	PfCK1α	LmCKI-2	TcCKI-2
TgCKIβ	100	48	48	45	44	42
TgCKIα		100	81	68	58	62
EtCKIα			100	67	59	60
PfCK1α				100	52	55
LmCKI-2					100	75
TcCKI-2						100

FIG. 5

A


Tg-α
Tg-β
Et-α

B


CK activity (cpm)
125000
100000
75000
50000
25000
0
Tg-α
Tg-β
Et-α
Mock

C

98
64
50
36
22

FIG. 6A-C

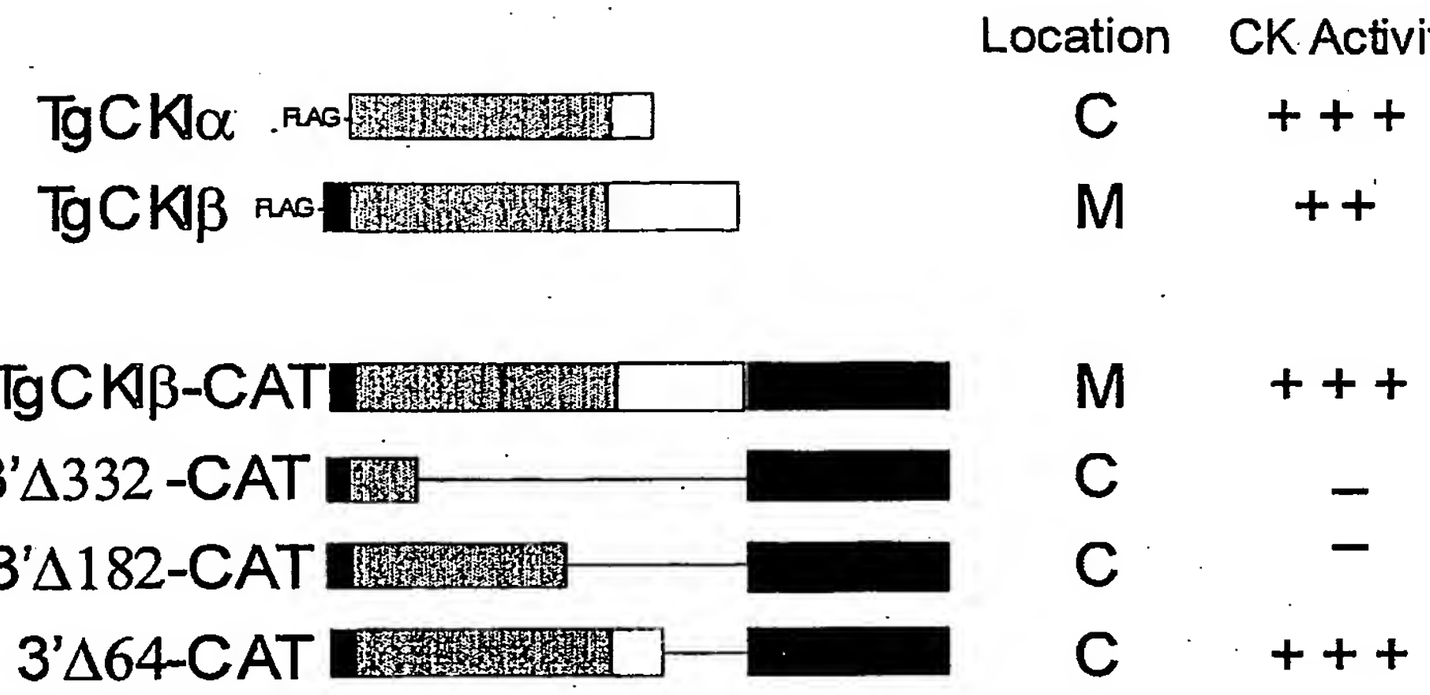
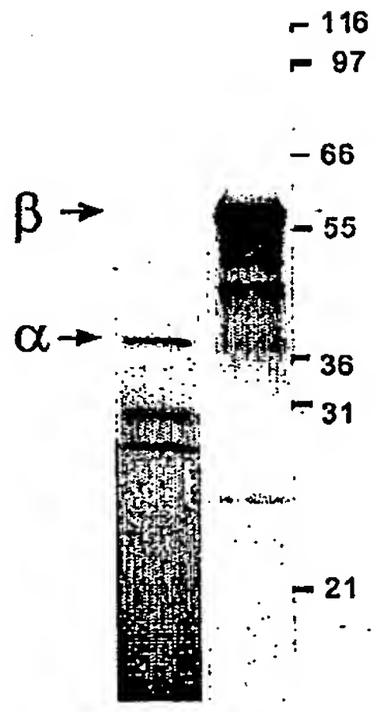

Location
CK Activity
TgCKIα
FLAG
C
+++
TgCKIβ
FLAG
M
++
TgCKIβ-CAT
M
+++
3'Δ332-CAT
C
–
3'Δ182-CAT
C
–
3'Δ64-CAT
C
+++

FIG. 7

A.


1 2
116
97
66
55
36
31
21
β
α

B.

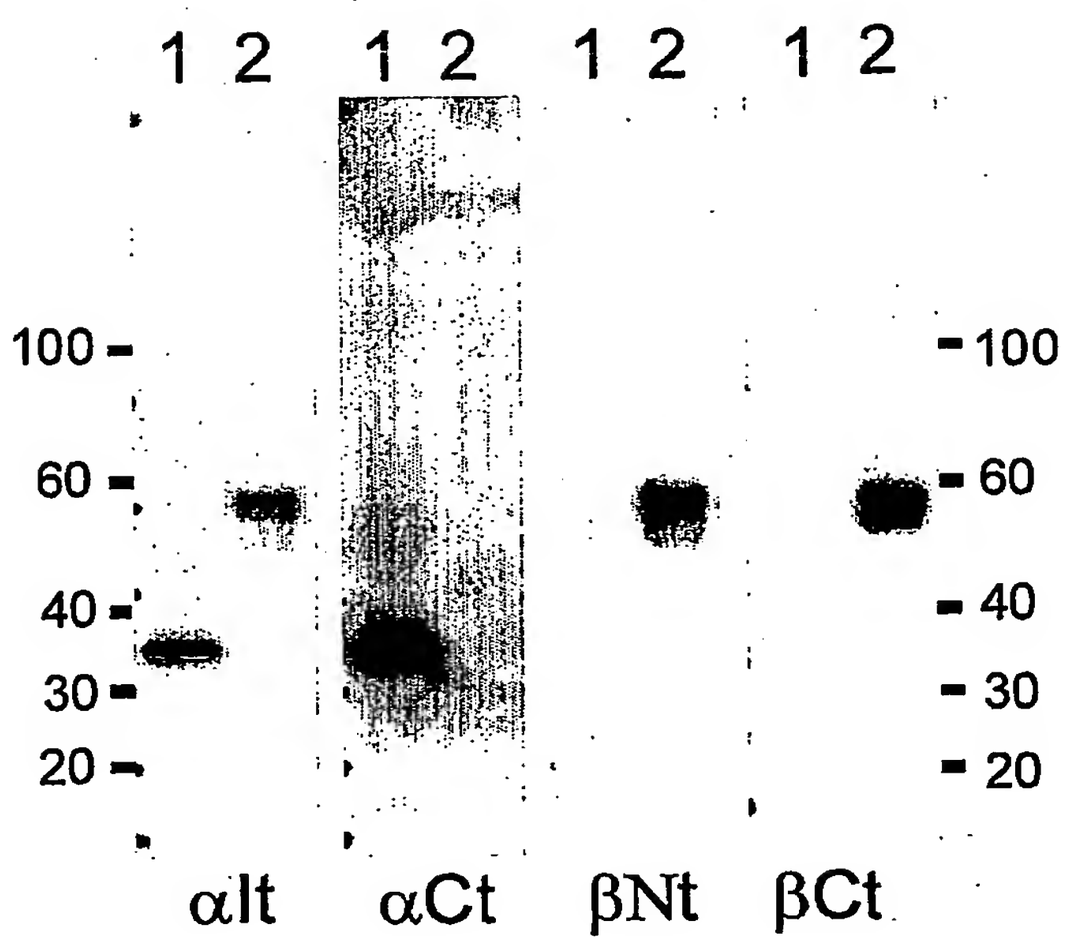

1 2 1 2 1 2 1 2
100
60
40
30
20
100
60
40
30
20
αIt αCt βNt βCt

FIG. 8A-B

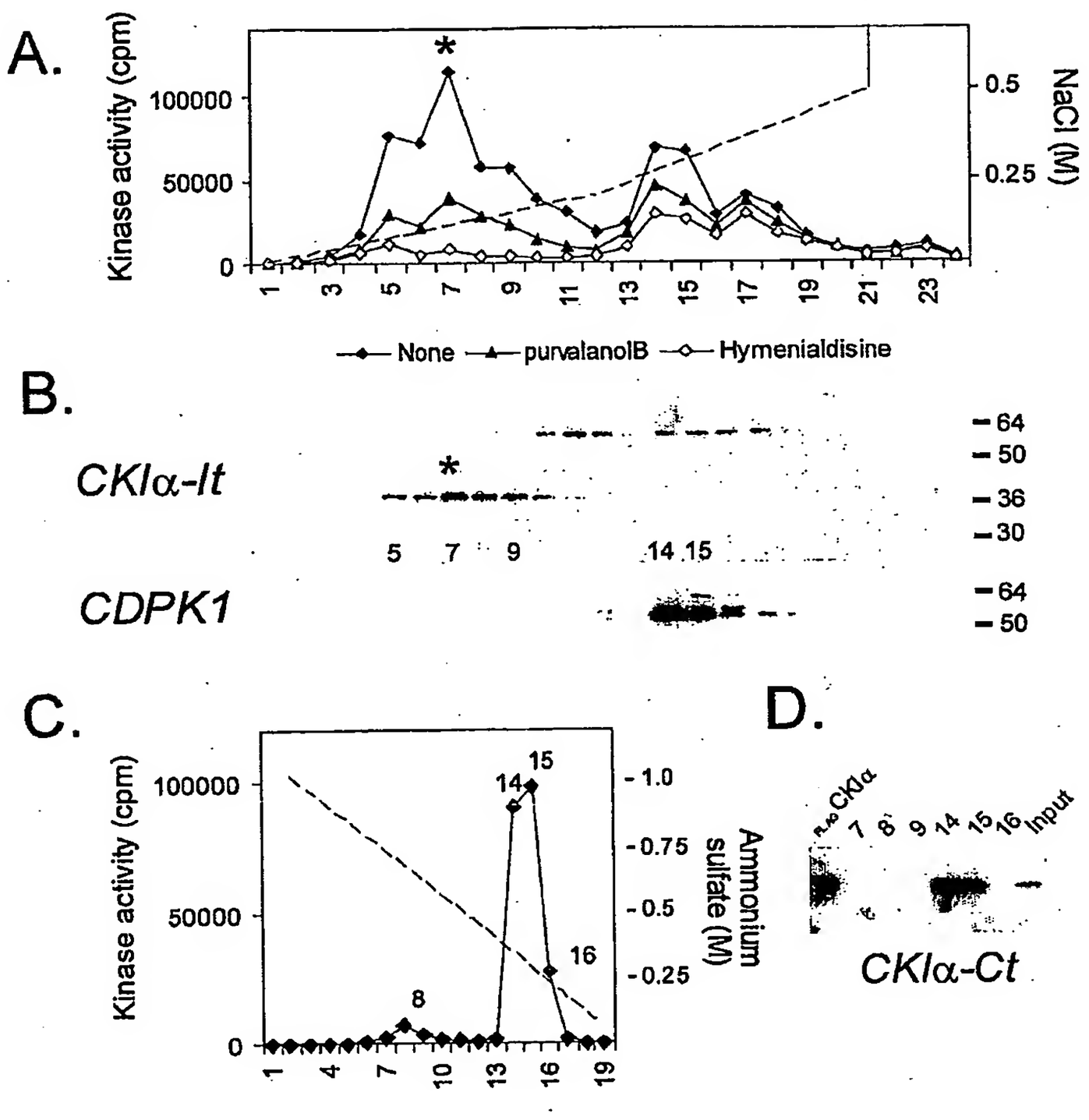
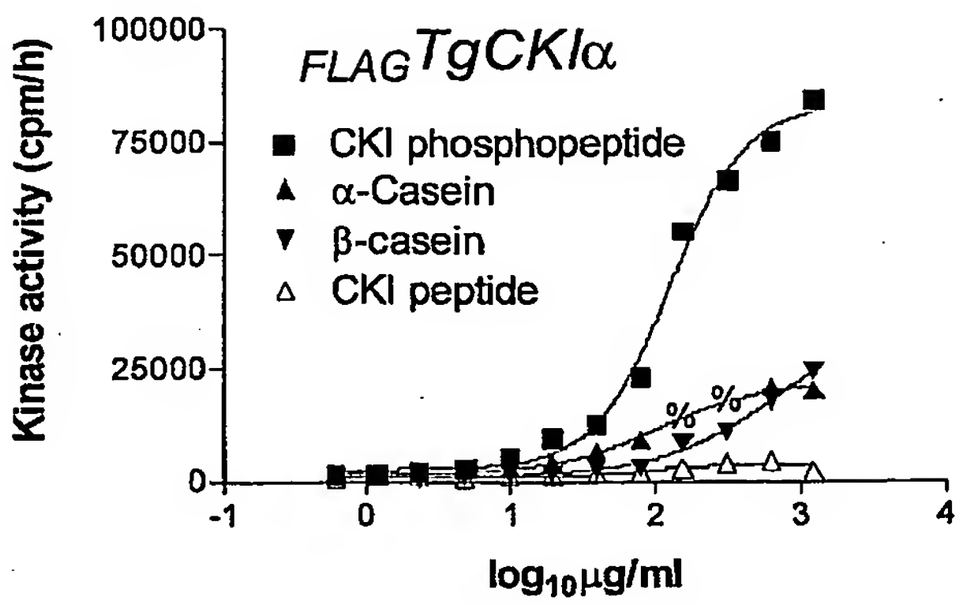
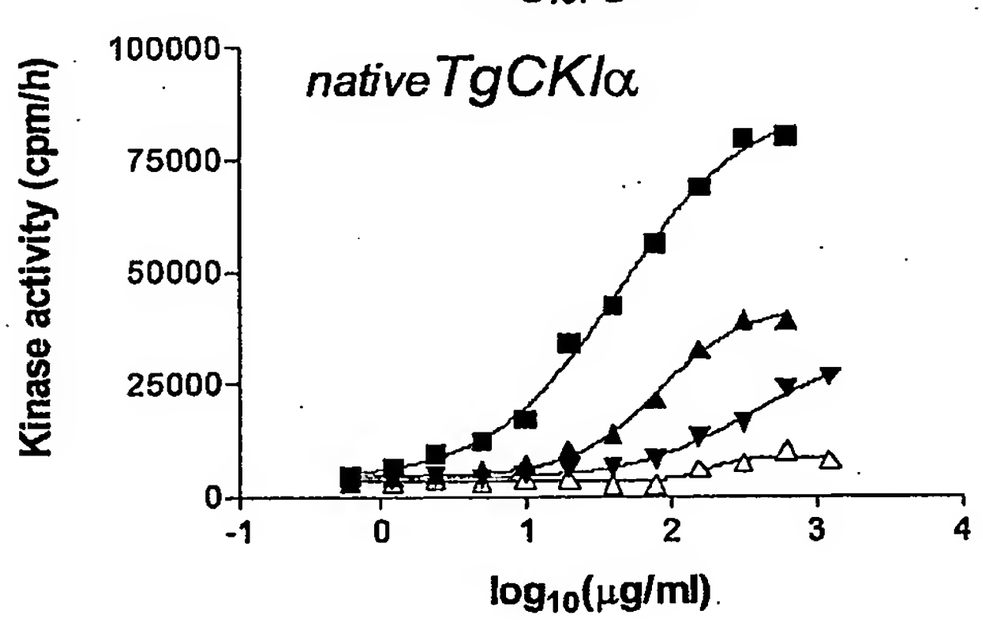

A.
Kinase activity (cpm)
100000
50000
0
*
0.5
0.25
NaCl (M)
1 3 5 7 9 11 13 15 17 19 21 23
None
purvalanolB
Hymenialdisine
B.
CKIα-It
*
5 7 9 14 15
64
50
36
30
CDPK1
64
50
C.
Kinase activity (cpm)
100000
50000
0
15
14
16
8
1.0
0.75
0.5
0.25
Ammonium sulfate (M)
1 4 7 10 13 16 19
D.
CKIα
7 8 9 14 15 16 Input
CKIα-Ct

FIG. 9A-D

A


FLAGTgCKIα
CKI phosphopeptide
α-Casein
β-casein
CKI peptide
Kinase activity (cpm/h)
100000
75000
50000
25000
0
-1
0
1
2
3
4
log10µg/ml

B


nativeTgCKIα
Kinase activity (cpm/h)
100000
75000
50000
25000
0
-1
0
1
2
3
4
log10(µg/ml)

C

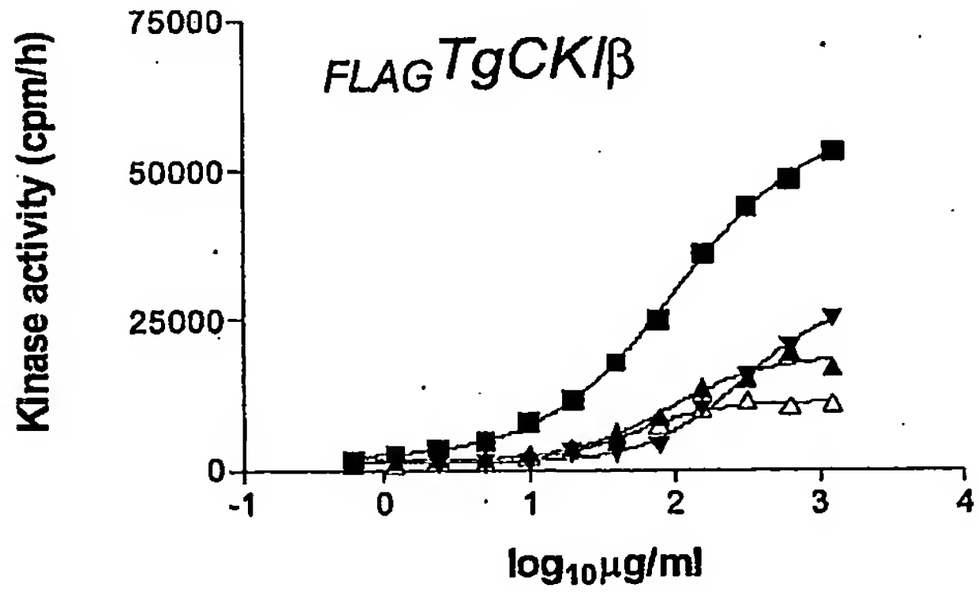
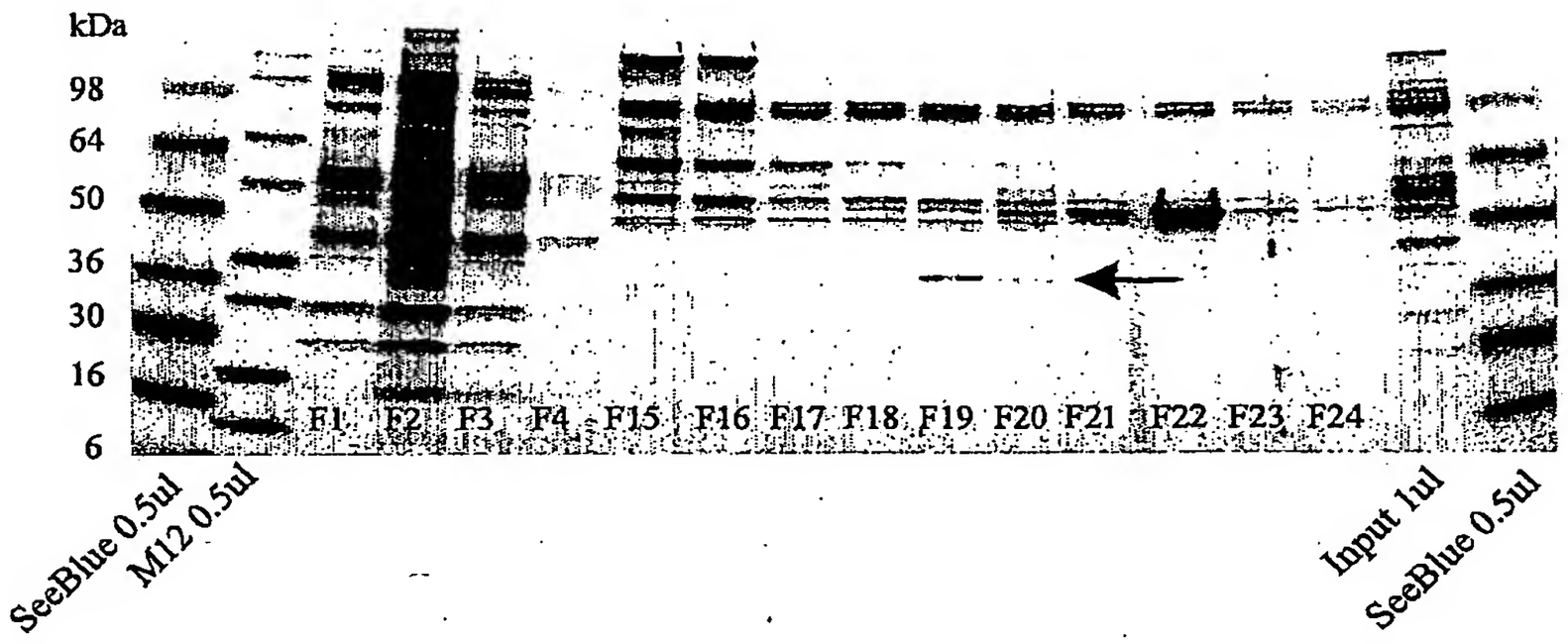

FLAGTgCKIβ
Kinase activity (cpm/h)
75000
50000
25000
0
-1
0
1
2
3
4
log10µg/ml

FIG. 10A-C

A.


kDa
98
64
50
36
30
16
6
F1
F2
F3
F4
F15
F16
F17
F18
F19
F20
F21
F22
F23
F24
SeeBlue 0.5ul
M12 0.5ul
Input 1ul
SeeBlue 0.5ul

B.

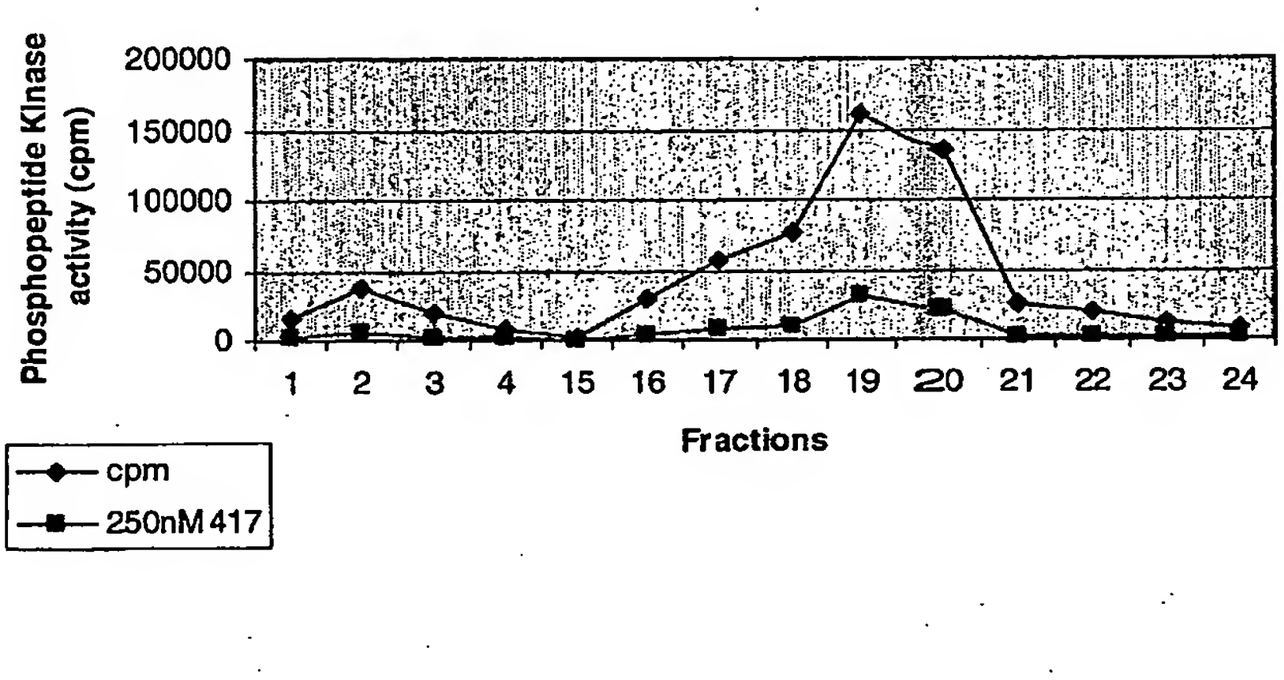

Phosphopeptide Kinase activity (cpm)
200000
150000
100000
50000
0
1
2
3
4
15
16
17
18
19
20
21
22
23
24
Fractions
cpm
250nM 417

FIG. 11A-B

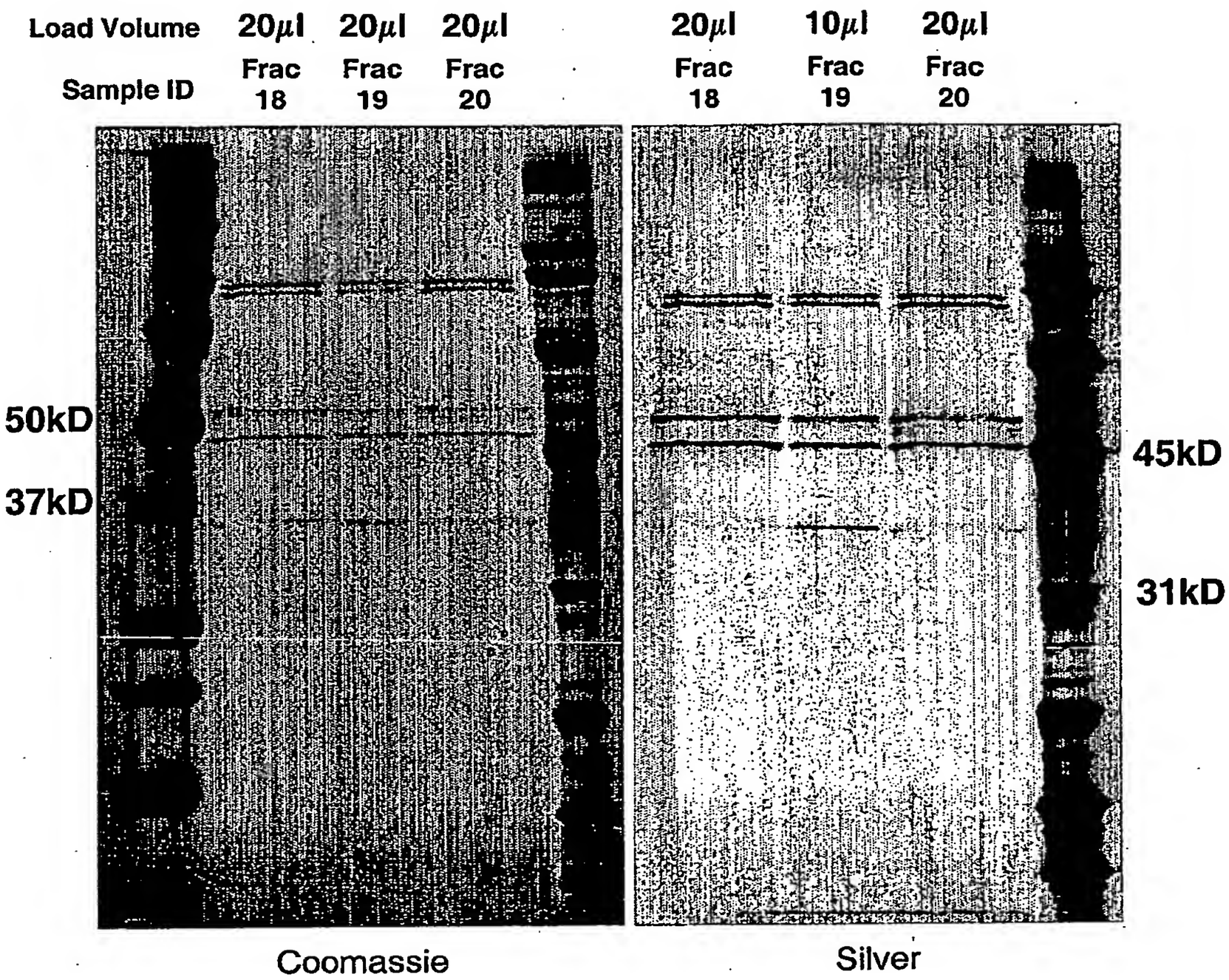

Load Volume
20µl
20µl
20µl
20µl
10µl
20µl
Sample ID
Frac 18
Frac 19
Frac 20
Frac 18
Frac 19
Frac 20
50kD
37kD
45kD
31kD
Coomassie
Silver

FIG. 12

MDVRVGGKYR LGRKIGSGSF GDIYLGTNIS TGDEVAIKLE
SVRSRHPQLI YESKLYKILT GGIGIPTLYW YGIEGDYNVM
IIELLGPSLE DLFSICNRKL SLKTVLMLAD QMLNRIEFVH
SRHFIHRDIK PDNFLIGRGK KMSIVFAIDF GLAKKYRDPR
TQSHIPYREG KNLTGTARYA SVNTHLGIEQ SRRDDLEALG
YVLMYFNRGS LPWQGLKATT KKDKYDKIME KKMSTPIEVL
CKQFPFEFIT YLNYCRSLRF EDRPDYSYLR RLFKDLFFRE
GYQYDFIFDW TFLHAERERE RQRRSMVNQG AESGNQWRRD
ASGRDPLGRL PQLEP (SEQ ID NO:2)

FIG. 13

	R_1, R_2
Purvalanol A	H, H
Purvalanol B	H, CO_2H
Aminopurvalanol	NH_2, H

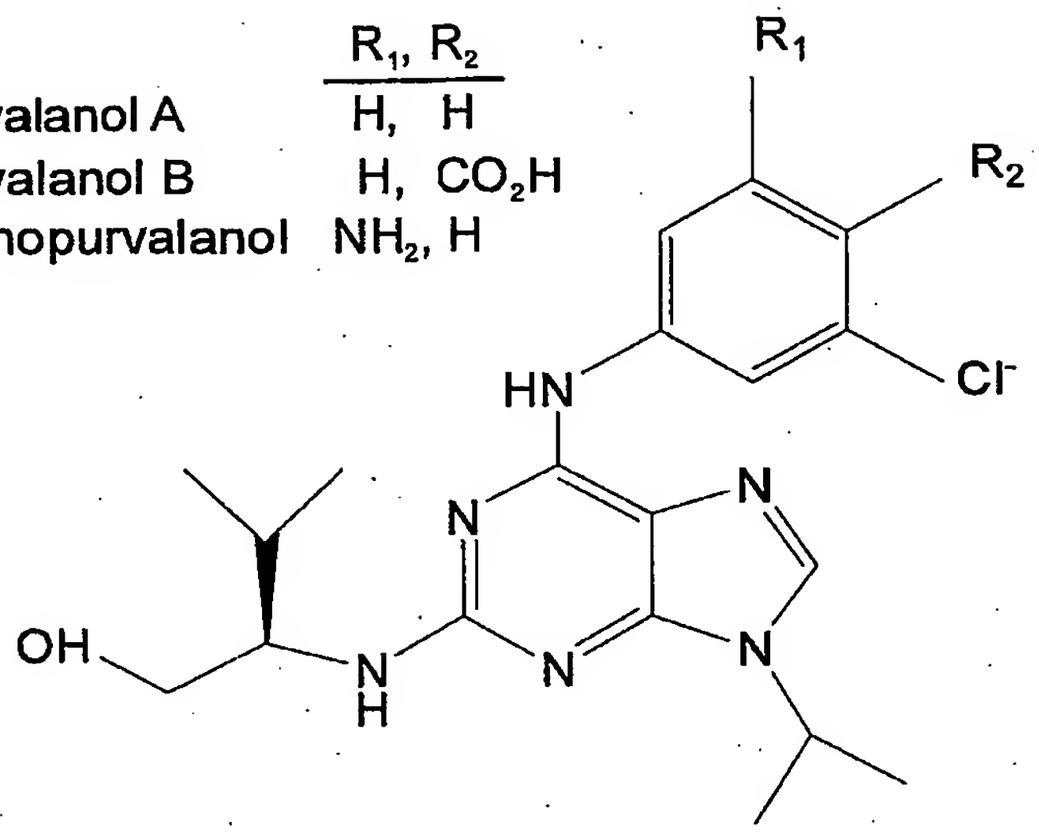


FIG. 14

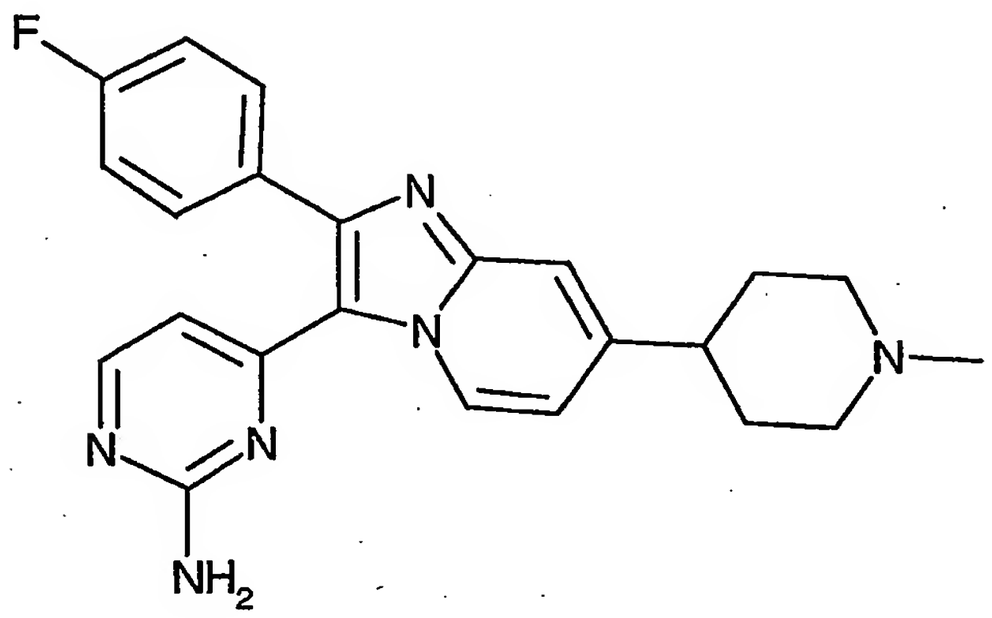


Fig. 15